RIDASCREEN® Chlamydia IgA

Enzyme immunoassay for the detection of
IgA antibodies against Chlamydia species in serum

Art. No.: K TB 3111

In vitro Test
Lagerung bei 2 - 8°C
Storage at 2 - 8°C

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Contents

1. Intended Use

The RIDASCREEN® Chlamydia IgA kit is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for the qualitative detection of IgA antibodies in human sera to Chlamydia antigen for the determination of immunological experience.

2. Introduction

The Chlamydiae are a group of obligate intracellular parasites with viral and bacterial characteristics (1,2). The order consists of three species, *Chlamydia trachomatis, Chlamydia pneumoniae* and *Chlamydia psittaci.*
C. trachomatis has been linked to urogenital infections (e.g., urethritis, epididymitis, cervicitis, PID), trachoma, infant pneumonia, and lymphogranuloma venereum (1,2). *C. pneumoniae* and *C. psittaci* are linked to pneumonia and respiratory disease (1,2,3).
Identification of Chlamydia directly in specimen material by culture, monoclonal DIFA, ELISA and nucleic acid amplification techniques are the most often used methods for the aid in diagnosis of *Chlamydia* infections. Serology may be helpful as additional information, providing indirect evidence of exposure, but may not correlate with infected status. Chlamydia serologies are not recommended for diagnosis of active disease except in suspected cases of LGV, psittacosis, and infants with pneumonia (IgM only); and commercial methods for the diagnosis of *C. trachomatis* have not been found to be reliable for the diagnosis of TWAR (*C. pneumoniae*) infection (4). Several ELISA assays for Chlamydia antibody have been described (5,6,7,8).
While direct antigen tests are ideal to detect infections of the lower genital tract, they fail in the case of silent chronic infections of the upper genital tract, which represent a frequent cause of female infertility. This is, by excellence, the field of investigation for serology. Due to sampling problems, they are of interest to investigate respiratory infection to one of the three Chlamydia species. The RIDASCREEN® Chlamydia IgA Enzyme-Linked Immunosorbent Assays (ELISA) can be used as an alternative to IgG testing, but these IgA antibodies appear later in the course of the disease than IgG antibodies. The Chlamydia trachomatis IgA antibody is closely associated with Chlamydia induced arthritis (including Reiter Syndrome) (16,17).

3. Principle of the Assay

The RIDASCREEN® Chlamydia Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e. antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgA conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Substrate/Chromogen tetramethyl-benzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is proportional to the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader (9,10,11,12).

4. Kit Presentation

Materials supplied

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Chlamydia trachomatis antigen (inactivated) coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant/humidity indicator.
2. Serum Diluent Type I: Ready to use. Contains proclin (0.1%) as a preservative, pH 7.5 ± 0.2. (30 ml)
3. Calibrator: Human serum or defibrinated plasma. Sodium azide (0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (0.4 ml) *
4. Positive Control: Human serum or defibrinated plasma. Sodium azide (0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (0.4 ml) *
5. Negative Control: Human serum or defibrinated plasma. Sodium azide (0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (0.4 ml) *
6. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgA, containing proclin (0.1%) and gentamicin as preservatives. (16 ml)
7. Substrate/Chromogen Solution: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (15 ml)
8. Washing Buffer (20x concentrate): Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and proclin (0.1%) as a preservative, pH 7.2 ± 0.2. (60 ml)
9. Stop Solution: Ready to use, contains a 1N H_2SO_4 solution. (15 ml)

* serum vials may contain excess volume

Note: Some components are not kit lot dependent and may be used interchangeably within the following RIDASCREEN® ELISA: Toxoplasma, Rubella, CMV and Chlamydia. Those components are: Washing Buffer, Substrate/Chromogen, Stop Solution. For IgA and IgG determination the Serum Diluent Type I has to be used. For IgM determination the Serum Diluent Type II has to be used.

Additional Requirements

1. Wash bottle, automated or semi-automated microwell plate washing system
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µl volumes (less than 3% CV)
3. One liter graduated cylinder
4. Paper towels
5. Test tube for serum dilution
6. Reagent reservoirs for multichannel pipettes
7. Pipette tips
8. Distilled or deionized water (dH_20), CAP (College of American Pathology) Type 1 or equivalent (18,19)
9. Timer (0 – 60 minutes)
10. Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950 ml dH_20)
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

5. Storage and Stability

1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant/humidity indicator and returned to storage between 2° and 8° C.
3. Store HRP Conjugate between 2° and 8° C.

4. Store the Calibrator, Positive Control, and Negative Control between 2° and 8° C.
5. Store Serum Diluent and 20x Washing Buffer between 2° and 8° C.
6. Store the Substrate/Chromogen Solution between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1x (diluted) Washing Buffer at room temperature (21° to 25° C) for up to 5 days, or up to one week between 2° and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination.

6. Precautions

1. For *in vitro* diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1 & 2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2 (13).
4. The components in this kit have been quality control tested as a master lot unit. Do not mix components from different lot numbers except the reagents named in the note of point 4. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25° C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.

8. Use only distilled or deionized water and clean glassware.

9. Do not let wells dry during assay; add reagents immediately after completing wash steps.

10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause false results.**

11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.

12. Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.

13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.

14. Never pipette by mouth or allow reagents or patient samples to come into contact with skin. Reagents containing proclin, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.

15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.

16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.

17. Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See precaution 3.

18. The concentrations of anti-Chlamydia trachomatis IgA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

7. Specimen Collection and Storage

1. Handle all blood, plasma and serum as if capable of transmitting infectious agents.

2. Optimal performance of the ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-lipemic, non-icteric). A minimum volume of 50 µl is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture (14). Early separation from the clot prevents hemolysis of serum.

3. Store serum between 2° and 8° C if testing will take place within two days. If specimens are to be kept for longer periods, store at –20° C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.

4. If paired sera are to be collected, acute samples should be collected as soon as possible after the onset of symptoms. The second sample should be collected 14 to 21 days after the acute specimen was collected. Both samples must be run in duplicate on the same plate to test for a significant rise. If the first specimen is obtained late during the course of the infection, a significant rise may not be detectable.

5. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990) (14).

8. Methods for Use

Preparation for the Assay

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.

2. All samples and controls should be vortexed before use.

3. Dilute 60 ml of the 20x Washing Buffer to 1.2 l with distilled and/or deionized H_20. Mix well.

Assay Procedure

Note: To evaluate paired sera, both serum samples must be tested in duplicate and run in the same plate. It is recommended that the serum pairs be run in adjacent wells.

1. Place the desired number of strips into a microwell frame. Allow four (4) Control/Calibrator determinations (one Negative Control, two Calibrators and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant/humidity indicator, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #4
1B	NC	2B	Patient #5
1C	Cal	2C	Patient #6
1D	Cal	2D	Patient #7
1E	PC	2E	Patient #8 (Acute 1)
1F	Patient #1	2F	Patient #8 (Acute 2)
1G	Patient #2	2G	Patient #8 (Convalescent 1)
1H	Patient #3	2H	Patient #8 (Convalescent 2)

RB = Reagent Blank - well without serum addition run with all reagents
NC = Negative Control
Cal = Calibrator
PC = Positive Control

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µl + 200 µl) in Serum Diluent Type I. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)

3. To individual wells, add 100 µl of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µl of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.

4. Incubate each well at room temperature (21° to 25° C) for 20 minutes ± 2 minutes.

5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µl of diluted Washing Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

IMPORTANT NOTE:

Regarding steps 5 and 8 - insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µl) is recommended. A total of up to five (5) washes may be necessary with automated equipment. Complete removal of the Washing Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 µl Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.

7. Incubate each well at room temperature (21° to 25° C) for 20 minutes ± 2 minutes.

8. Repeat wash as described in Step 5.

9. Add 100 µl Substrate/Chromogen Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.

10. Incubate each well at room temperature (21° to 25° C) for 10 minutes ± 2 minutes

11. Stop reaction by addition of 100 µl of Stop Solution (1N H_2SO_4) following the same order of Substrate/Chromogen addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. Wait a minimum of 5 minutes and read. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is ≥ 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

9. Quality Control

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each test run.

2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.

3. Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).

4. Each Calibrator must be ≥ 0.250 A at 450 nm (when read against reagent blank).

5. Positive Control must be ≥ 0.500 A at 450 nm (when read against reagent blank).

6. The ISR (Immune Status Ratio) Values for the Positive and Negative Control should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and should be repeated.

7. Additional Controls may be tested according to guidelines, or requirements of local, state, and/or federal regulations or accrediting organizations.

8. Refer to NCCLS C24-A for guidance on appropriate QC practices (15).

10. Interpretation

Calculations

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value from the two Calibrator determinations.

2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined for each lot of kits. The Correction Factor is printed on the Calibrator vial.

3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.

4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3.

Example:

O.D.'s obtained for Calibrator	= 0.38, 0.42
Mean O.D. for Calibrator	= 0.40
Correction factor	= 0.50
Cutoff Calibrator Value	= 0.50 x 0.40 = 0.20
O.D. obtained for patient sera	= 0.60
ISR Value	= 0.60/0.20 = 3.00

Analysis

1. The patients' ISR (Immune Status Ratio) values are interpreted as follows:

ISR	Results	Interpretation
≤ 0.90	Negative	No detectable antibody by the test. Such individuals are presumed to be uninfected and to be susceptible to primary infection.
0.91-1.09	Equivocal	Samples should be retested. See Number (2) below.
≥ 1.10	Positive	Indicates presence of detectable antibody by ELISA test. Indicative of current or previous infection. The individual may be at risk of transmitting infection, but is not necessarily currently contagious.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.

3. In the evaluation of paired sera, if the acute specimen is negative and the convalescent specimen is positive, a seroconversion has taken place. This indicates a significant change in antibody level and the patient is undergoing a primary infection.

4. To evaluate paired sera for a significant change in antibody level or seroconversion, both samples must be tested in duplicate in the same assay. The mean ISR of both samples (acute and convalescent) must be greater than 1.00 to evaluate the paired sera for significant rise in antibody level.

5. Additional Quality Control for Paired Sera: (See NOTE under Assay Procedure). As a check for acceptable reproducibility of both the acute sera (tested in duplicate) and the convalescent sera (tested in duplicate), the following criteria must be met for valid results:

$$\frac{\text{Acute 1 ISR}}{\text{Acute 2 ISR}} = 0.8 \text{ to } 1.2 = \frac{\text{Convalescent 1 ISR}}{\text{Convalescent 2 ISR}}$$

6. Compare the ISR of the pairs by calculating as follows (% rise in ISR level):

$$\frac{\text{Mean ISR (second sample) - Mean ISR (first sample)}}{\text{Mean ISR (first sample)}} \times 100$$

% rise in ISR	Interpretation
< 30.0 %	No significant change in antibody level. No evidence of recent infection. If active disease is still suspected, a third sample should be collected and tested in the same assay as the first sample to look for a significant rise in antibody level.
≥ 30.0%	Statistically significant change in antibody level detected. This identifies those persons who are presumed to be experiencing recent or current episodes of infection (reactivation, reinfection or a primary infection where the acute specimen was obtained too late to demonstrate seroconversion).

Note: When evaluating paired sera, it should be determined if samples with high absorbance values are within linearity specifications of the spectrophotometer. Read the Operator's Manual or contact the instrument's manufacturer to obtain the established linearity specifications of your spectrophotometer.

11. Expected Values

The expected values from the performance of this kit has not been established. See Table 1 for a listing of published Clamydia infections (16,17).

Table 1: Chlamydia Infections

Etiologic Agent	Strains	Clinical Manifestations	Epidemiology
C. trachomatis	Serovars A, B, BA, C	Trachoma	rare in Europe, common in North America and Southeast Asia
	Serovars D, E, F, G, H, I, J, K	Genital infections -urethritis -cervicitis -epididymitic -acute salpingitis -inguinal adenopathy	common MST, major cause of female sterility
		Inclusion conjunctivitis	in adults or in newborns, secondary to genital infection (12% of cases)
		Pneumonia	in newborns from infected mothers
		Systemic complications -arthritis	in 10% of C. trachomatis urethritis, rarely isolated (2%) or as part of a Reiter Syndrome (8%)
		-heart, liver	rare
	Serovars L1, L2, L3	Lymphogranuloma venereum (LGV)	relatively uncommon in Europe, MST currently under-recognized , important cause of proctocolitis in homosexual men
C. psittaci	Multiple serovars	Pneumonia with systemic complications	uncommon occupational disease (poultry industry, exposure to exotic birds), transmission by the aerosol route from infected litter
C. pneumoniae	TWAR strains	Pneumonia, pharyngitis	probably common

12. Limitations of Use

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
2. False positive results may occur with sera from patients who have had other bacterial infections. Cross-reactivity of this assay with antibodies to other microbial agents has not been determined.
3. A negative antibody result does not rule our current or past infedtion. False negative results may occur when samples are drawn too early after infection/exposure. Production of detectable IgA antibody levels may be delayed. Some patients may never generate detectable antibody levels. Patients with symptoms suggestive of Chlamydia infection should be tested using direct methods such as culture, DFA, other antigen detection methods or detection of nucleic acids. Due to the fact that chlamydial infections are common and antibody may persist after infection, chlamydial antibody is often detected in healthy individuals.
4. The RIDASCREEN® Chlamydia IgA ELISA uses a broadly reactive Chlamydia antigen (LGV II (mainly Lipopolysaccharide)) that cannot differentiate between the different species of Chlamydia. Patients may be infected or exposed and have a serological response to one or more species of Chlamydia.
5. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
6. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.

13. Performance Characteristics

The performance characteristics of the RIDASCREEN® Chlamydia IgA ELISA test kit have not been established.

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